

***Ex-vivo* antihypertensive and calcium channel blocking activity of *Androsace foliosa* n-hexane leaves fraction on isolated rabbit aorta**

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Abstract: Hypertension is persistent elevation in blood pressure for 3-4 weeks. Estimated global prevalence of hypertension suggested that by the Year 2025 (29%) of adult worldwide are suffering from hypertension (1.56 billion). Hypertension complications are hemorrhage, atherosclerosis, renal artery stenosis, angina pectoris end organ damage, cardiomyopathy, myocardial infarction and retinopathy. Along with other drug class Calcium channel blocker are also used for the treatment of hypertension. In this study the possible action of the n-hexane leaves fraction of the *Androsace foliosa* on isolated rabbit aorta was examined. Antihypertensive activity was examined in the existence of standard agonist like phenylephrine and antagonist like Verapamil. Phenylephrine (PE 1 μ M) high K⁺ was used to steady the tissue materials. Additionally to observe the calcium channel blocking effect the tissues were treated with n-hexane segment of *A. foliosa* leaves. Aortic tissues were treated 4-5 intervals with Ca⁺²- free preparation earlier to control calcium reaction curve (CRCs). Verapamil is utilized as standard calcium channel inhibitory mediator and is used as an antagonist. The *A. foliosa* n-hexane leaves fraction completely inhibited the precontractions induced by Phenylephrine (1 μ M) and K⁺ (80 mM) precontractions, with EC₅₀ standards of 1.0mM (0.3-1.0mg/mL) and 4.90mM (1-3mg/mL), respectively. *Androsace foliosa* n-hexane leaves fraction was tested for calcium channel inhibitory effect on isolated rabbit aorta. *A. foliosa* n-hexane leaves segment at the dosage of 1mg/mL block the calcium channel approximately (35 \pm 5%). Consequence indicates that *A. foliosa* n-hexane leaves segment block calcium channel in the similar manner as compared to the standard calcium channel blocker drug (verapamil).

Keywords: *Androsace foliosa*, hypertension, phenylephrine, verapamil. calcium channel blocker.

INTRODUCTION

Raised blood pressure is also called hypertension (HBP) means the pressure in arteries is higher than normal. Hypertension is termed as consistent raise in blood pressure for at-least 2-4 weeks. Hypertension is also known as silent assassin because commonly it is asymptomatic (Sembulingam and Sembulingam 2012). Hypertension is also due to elevated peripheral vascular arteriolar smooth muscles pitch which results in increased arteriolar resistance. Additionally, hypertension also results from reduced capacitance of the venous system. Mostly increased tone of vascular system is unknown. Major sign and symptoms of hypertension are blurred vision, confusion, irregular heart beat and shortness of breath. Patients with hypertension also experience headaches, faintness, dizziness, tinnitus and changed vision (Giles, Materson *et al.*, 2009). Hypertension is classified according to the recommendations of seventh

Joint National Committee (JNC-7).

Hypertension is categorized in two chief types according to etiology i.e. primary and secondary. Firstly class of hypertension is classified as primary or essential hypertension and is responsible for 95% of total hypertension (Gifford Jr 1988). Primary hypertension is idiosyncratic in nature. The primary hypertension is more probably advances in patients with a diversity of threat factors. These risk causes include Black race, family history of hypertension, dyslipidemia, alcohol ingestion obesity and the personal approach (Burt, Whelton *et al.*, 1995). Secondly, hypertension is classified as secondary or non-essential hypertension and accounts for only 5% of total hypertension. While, secondary hypertension possess numerous known causes and onset is usually 25-55 years. It is less developed in persons less than 25 years of age. Causes of secondary hypertension include primary renal disease, reno-vascular disease, endocrine disorders, pheochromocytoma, cushing syndrome and primary Aldosteremia (Shafi and Shafi 2017).

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Hypertension is greater threat factors for numerous vascular diseases e.g. coronary heart diseases, cardiac failure (CF), stroke and end stage renal failure. Additionally, hypertension possesses numerous complications that are life threatening (Gifford Jr 1988). These complications are hemorrhage, atherosclerosis, renal artery stenosis, end organ damage, angina pectoris, myocardial infarction and retinopathy (Midaoui and de Champlain 2002). So prevention of hypertension is important factor to manage above mentioned complications. There are many elements which cause hypertension and these are categorized as non-modifiable and modifiable threat elements correspondingly (Gifford, 1988).

Classes of drugs that are used as an antihypertensive therapy includes diuretics, alpha and beta blockers, ACE inhibitors, angiotension receptors blockers (ARBs), rennin inhibitors, calcium channel blocker, aldosterone antagonists and centrally acting vasodilators (Fisher 2005).

Estimated global prevalence of hypertension is high long term projections suggested that by the Year 2025 (29 %) of adult worldwide are suffering from hypertension (1.56 billion). Data from 2011-2014 indicates that 46% of adults 18 year and older in United States are suffering from hypertension. Approximately, 103 million adults are suffering with hypertension (Thornton, Tooher *et al.*, 2016). In all WHO provinces, men have marginally greater frequency of hypertension than women. Global frequency of hypertension was 1.39 billion individuals. Among 31% of all adults this characterized 5.2% rise in the global prevalence in the year 2000-2010. The overall occurrence of hypertension was 26%; the prevalence amongst males (34%) was greater than females (24%) (TARAZI, Miller *et al.*, 1966; Sacks and Campos 2010). In Pakistan the average 3 or more medicines are prescribe per patient in case of hypertension. In private sector average of prescribed medicines per patient are 4.1. Meanwhile for hypertension in public sector average of drug prescribed per patient are 2.7 respectively (Healey and Connolly 2003).

Medicinal and economic importance

Androsace foliosa grows wildly in Kashmir, Hazara and Murree hills of Pakistan. This plant is called as common rock jasmine in English and Andros in Urdu. Meanwhile vernacular name of this plant is Thandi booti. Plant is locally used to treat liver diseases e.g., hepatic coma, peptic ulcer and also for kidney and renal diseases especially for treatment of kidney stones and pyelonephritis. Leaves are used for correcting menstrual flow, and helping avoiding conception (Boles *et al.*, 2015). *Androsace foliosa* is also used to cure amenorrhea, skin allergies, leucorrhoea and as abortifacient. The

different classes of phyto-chemicals reported from *Androsace* species include saponins and sapogenins (Sacks & Campos., 2010). Ethno-pharmacologically this plant is used for hypertension but scientifically the anti-hypertensive action was not confirmed. The present study was conducted on confirm the anti-hypertensive effect of this medicinal herb. Additionally calcium channel blocking effect was also performed to further confirm the exact mechanism of anti-hypertensive action of this herb.

MATERIALS AND METHODS

Plant accumulation

Herb *Androsace foliosa* was collected from Donga Gale, Ayyuba National Park Pakistan in July 2014. The plant was identified by a Botanist (Taxonomist) and the specimen is kept in herbarium of Botany Dept G.P.G.C # 1. After washing carefully with tap water to remove dirt the plant was shade dried. After drying plant was chopped and reduced to fine powder. Plant in the form of fine powder was kept in air tight container. Fresh plant was 5kg and after drying it weighed 2 Kg.

Preparation of fraction

Shade drying method was selected for drying of leaves of *Androsace foliosa*. After drying the leaves were pulverized with the help of electric grinder. After pulverization maceration process was adopted for extraction process. For extraction purpose leaves powder (500 grams) and soaked in n-hexane (1500ml) for total of 7 days. Intermittent shaking was also performed during the extraction process. The blend was filtered with the help of muslin cloth afterwards by the help of Whatman filter paper (No.1). The resultant filtrate was evaporated to dryness with the help of rotary evaporator under decreased pressure. After dryness the fraction provide a yield of 11 percent (w/w).

Animals

Virgin female rabbit weight (1-2kg) was used for the study. The animals were kept at standard condition. (Temperature $21\pm 3^{\circ}\text{C}$, sultriness 30-70% and 12hr light and 12hr dark rhythm) in Animal center organization of pharmacy and technology laboratory Abbottabad. They have excess to regular diet and un-limited excess to water. The animals were fasted 24 hours before the experiment. The research was performed with appropriate authorization by organization Animal Ethics board. Experiments were conducted according to the modern guiding principle for the concern of laboratory animals.

Drugs and chemicals

Phenylephrine, high K^+ and Verapamil are drugs used in current study. Chemicals used were NaCl, NaHCO_3 , CaCl_2 , KCl, KH_2PO_4 , MgSO_4 and glucose. All the chemicals were obtained from Merck Germany.

Tissue preparation and experimental procedure

The rabbits were executed by cervical displacement; the thoracic aorta was detached and changed into portions of almost 2-3 mm thickness. Aortic rings were adjoined among a couple of stainless steel knobs in 10mL tissue bath. 1 knob was attached to a steel bar at the end and former was festinated to power transducer (MLT) 0201 coupled to Power Lab Data Acquisition structure. Tissue was kept in standard Krebs's solution, kept at 37°C and ventilated uninterruptedly through carbogen (95% O₂ and 5% CO₂). Ingredients of Krebs's solution were (mM): NaCl 118.2g, NaHCO₃ 25.0g, CaCl₂ 2.5g, KCl 4.7g, KH₂PO₄ 1.3g, MgSO₄ 1.2g glucose 11.7g). Furthermore, pH was retained at 7.4. Pre load of 2g was provided to every tissues and stability period of 60 mints was endorsed for merely inspecting the postulation of experimental constituents. Phenylephrine (PE 1μM) was utilized to balance the trial tissues. Fluctuations in isometric tightness was documented and evaluated using a Force Transducer (MLT-0201), Connect with a channel Amplifier (N12128) and Power Lab (ML 846) Data Acquirement Structure (AD Instruments, Sydney, Australia) (Hassan Gilani, Khan *et al.*, 2005).

Outcome on Pre-contracted rabbit aortic rings

Phenylephrine (1μM) and K⁺ were utilized to produce steady-state shrinkages of animal aorta. The *Af.* n-hexane leaves fraction was added in an accumulative manner to attain concentration reaction turns. Furthermore, relaxation was quantified as percentage of agonist-persuaded tightening. These procedures confirmed that there is a secondary method to discover consequence of the *Af.* n-hexane leaves segment on voltage-operated (VDCs) and receptor-functioned Ca²⁺ networks (ROCs) and Ca²⁺ discharges from internal stock(s).

Calcium Network blocking action

In a series of trials, an strength was prepared to proclaim if the reduction influenced by the *A. foliosa* n-hexane leaves fraction fashioned Ca²⁺ entry by VDCs. Aortic rings were washed for 4-5 intervals with Ca²⁺- free solution previously the control calcium reaction curvature (CRCs). After the controller CRCs of Ca²⁺ were found super-imposable, formerly tissue was pretreated with *A. foliosa* n-hexane leaves fraction for 30 to 45 minutes to check the probable calcium channel inhibitory consequence. Analogous control was also tested under parallel investigational circumstances.

STATISTICAL ANALYSIS

Results for the parametric tests were expressed as mean ± standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) were acclimated to determine statistical paramountcy and followed by Tukey-Kramer or Dunnett's post-test. Fisher's exact test was utilized for frequency comparison.

RESULTS

Phenylephrine (1 μM) and K⁺ (80 mM) produce stable shrinkages in separated rabbit aortic rings, in which a collective accumulation of *A. foliosa* n-hexane leaves

fraction persuaded a vasodilator activity alongside both contractile stimulants. Preincubation of aortic tissues with *A. foliosa* n-hexane leaves fraction (0.1-1.0mg/mL) moved the Ca²⁺ CRCs to the right fashioned in Ca²⁺-free solution. The *A. foliosa* n-hexane leaves fraction completely inhibited the precontractions induced by Phenylephrine (1 μM) and K⁺ (80 mM) precontractions, with EC₅₀ values of 1.0mM (0.3-1.0mg/mL) and 4.90mM (1-3mg/mL), respectively (fig. 1). Verapamil calm the PE (1μM) and K⁺ (80mM)-induced contractions with EC₅₀ value of 0.4mM and 0.8mM, correspondingly (fig. 2). Results indicate that *A. foliosa* n-hexane leaves fraction was more specific towards K⁺ (80mM)-persuaded contraction as equated to PE. This curve displaying the dose-dependent blocking action of *A. foliosa* n-hexane leaves segment and verapamil (1μM) on K⁺ and phenylephrine (PE)-induced contractions in separated rabbit aorta tissues. The signs symbolize mean & standard inaccuracy of mean n-4.

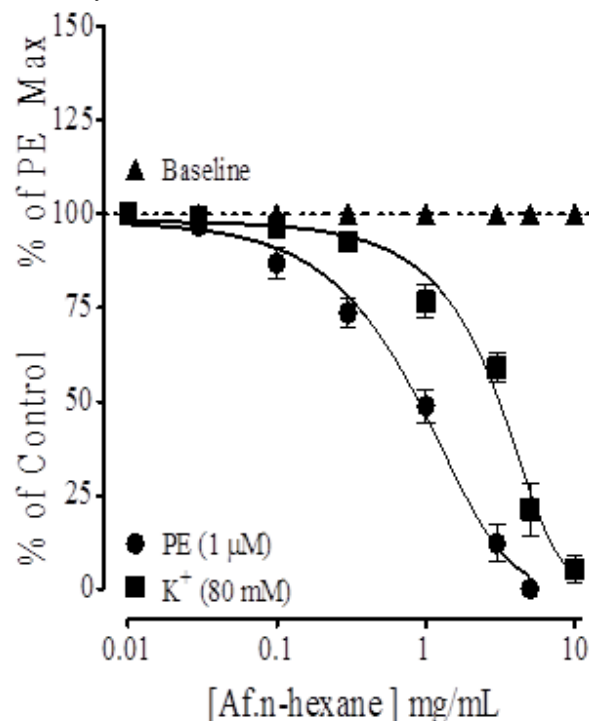


Fig. 1: Outcome of *A. foliosa* n-hexane leaves segment on Isolated Rabbit aorta in the presence of Phenylephrine (1 μM) and elevated K⁺ (80mM)-produced contraction.

A. foliosa n-hexane leaves fraction was tested for calcium channel blocking effect on isolated rabbit aorta. A dose dependent blocking effect was observed with increasing doses of *A. foliosa* n-hexane leaves fraction. Calcium channel blocking effect was observed with the *Af.n-*

Table 1: Indicate the different stages of hypertension (Muntner, Carey et al. 2018)

Blood pressure grouping	Systolic (mm Hg)	Diastolic (mm Hg)
Standard	≤ 120	≤ 80
Raised	120-129	Greater than 80
Stage I Hypertension	130-139	80-89
Stage II Hypertension	140 or greater	90 or greater
Hypertensive crisis	Greater than 180	Greater than 100
Malignant hypertension	Greater than 200	Greater than 110

hexaneleaves fraction at doses of (1mg/mL, 3mg/mL and 5mg/mL) and compared with control (100%). The *A. foliosa* n-hexane leaves segment at the dosage of 1mg/mL block the calcium channel approximately (35±5%), and at the dosage of 3mg/mL block almost (55±5%) and at 5mg/mL block (70±5%) of the calcium channel respectively (fig. 3). Outcome indicates that *A. foliosa* n-hexane leaves segment block calcium channel in the similar manner as compare to the standard calcium channel blocker drug (verapamil) (fig. 4). Dose response curve showing the dose-dependent curve of Ca⁺⁺ in the existence of collective dosage of the *A. foliosa* n-hexane leaves fraction and standard drug verapamil induced-contraction on calcium curve on separated rabbit aortic tissues (values are shown as mean ± S.E, n=5).

DISCUSSION

Hypertension is predictable to cause approximately 12.8 % of total deaths and 7.5 million deaths annually. Increased blood pressure is chief threat element for coronary heart malady, hemorrhagic and ischemia stroke. Existing treatment regime is effective in management of hypertension, but with some limitations e.g. side effects and availability. Apart from coronary heart disease and stroke, complication of elevated blood pressure comprised of heart failure, exterior vascular disease, visual and renal damage and retinal damage (Go, Mozaffarian *et al.*, 2013).

Application of *A. foliosa* n-hexane leaves fraction to uninterrupted rabbit aortic fragments not caused in alteration of baseline tension, signifying that this segment has no contractile activity under relaxing situation. The result of study clearly shows that the *A. foliosa* n-hexane leaves fraction induce lessening of rabbit aortic smooth tissue resulting pre-contraction produced by phenylephrine or elevated K⁺. The two contractile mediators are recognized to bring vascular smooth muscle tightening by two distinct methods. Firstly, phenylephrine brings contraction by triggering α-adrenergic receptors on the vascular smooth muscle tissue causing in Ca²⁺entrythrough receptor-activated Ca²⁺networks as well as Ca²⁺since intracellular storing locations.

Oppositively elevated K⁺ tightening comprises membrane depolarization and stimulation of Ca²⁺entry via potential complex networks. Phenylephrine (1 μM) and K⁺ (80 mM) created stable contractions in separated rabbit aortic segments. Accumulative of *A. foliosa* n-hexane leaves segment produced a vasodilator action beside both contractile agents. Pretreating of aortic muscles with *A. foliosa* n-hexane leaves segment (0.1-1.0mg/mL) vary the Ca²⁺ CRCs to the right-side assembled in Ca²⁺-free solution. *A. foliosa* n-hexane leaves fraction completely inhibited the precontractions induced by Phenylephrine (1 μM) and K⁺ (80 mM) precontractions, with EC₅₀ values of 1.0mM (0.3-1.0mg/mL) and 4.90 mM (1-3mg/mL), respectively (fig. 1). Verapamil relaxed the PE (1μM) and K⁺ (80mM)-induced contractions with EC₅₀ value of 0.4mM and 0.8mM, respectively, (fig. 2). Results indicate that *A. foliosa* n-hexane leaves fraction was more specific towards K⁺ (80 mM), induced contraction as compared to PE.

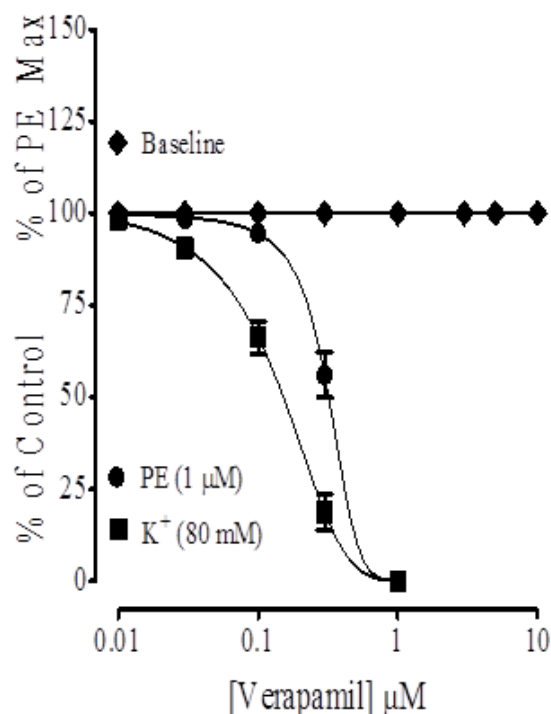


Fig. 2: The effect of *A. foliosa* n-hexane leaves fraction and standard drug verapamil induced- contractions on standard calcium curve on isolated rabbit aorta.

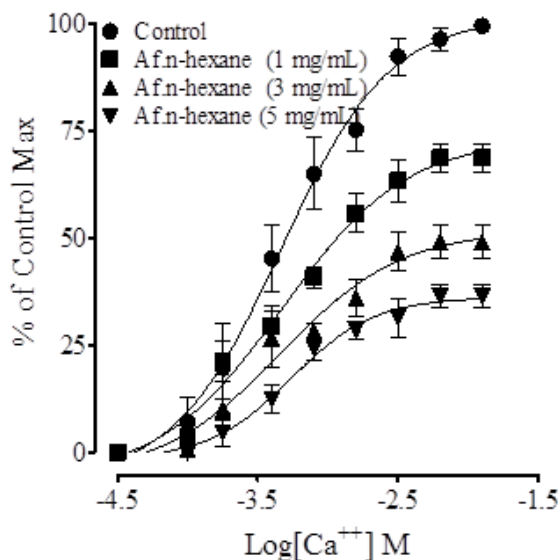


Fig. 3: Effect of different doses of *A. foliosa* n-hexane leaves fraction and induced- contractions on standard calcium curve on isolated rabbit aorta.

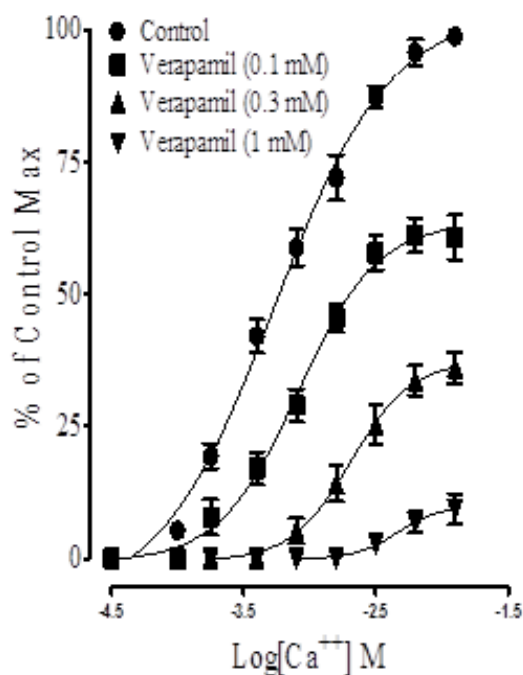


Fig. 4: Effect of different doses of *A. foliosa* n-hexane leaves fraction and standard drug verapamil induced-contractions on standard calcium curve on isolated rabbit aorta.

The calcium channel inhibitors are the assorted set of anti-hypertensive drugs. Primary outcome of calcium network blockers is to lessen the degree of access of calcium into the cytoplasm. In vascular smooth muscle strength this effect results in reduced cytosolic calcium concentration and reduction in contractile activity

resulting in vasodilation, range of calcium network blockers have verified in the management of hypertension and general vasodilator action. Different medicinal plants have shown calcium channel blocking effect e.g. *Ligusticum striatum*, *Uncaria rhynchophylla*, *Curcuma longa*, and *Ginger officinalis* (Hamilton 2004). *Af.n-hexane*leaves fraction was tested for calcium channel blocking effect on isolated rabbit aorta. A dose related calcium channel inhibitor action was detected with collective doses of *Af. n-hexane*leaves fraction. Calcium channel inhibitory effect was observed with the *Af.n-hexane*leaves segment at doses of (1mg/mL, 3mg/mL and 5mg/mL) and matched with control (100%). *Af.n-hexane*leaves segment at the dosage of 1mg/mL block the calcium channel approximately (35±5%), at the dose of 3mg/mL block almost (55±5%) and at the dosage of 5mg/mL block (70 ±5%) of the calcium channel respectively (fig 3). Results show that *A. foliosa* n-hexane leaves fraction block calcium channel in the similar manner as compared to the standard calcium channel blocker drug Verapamil (fig. 4). Results of the study clearly indicate that *A. foliosa* n-hexane leaves fraction is a potent calcium channel blocker. Further work is needed to confirm its precise mechanism of calcium channel blocking effect and molecule responsible for such activity.

CONCLUSION

Hypertension is sustained elevation in blood pressure. Countless peoples are suffering from hypertension and it causes numerous complications. Modern drugs contain side effects and are costly. The plant *Androsace foliosa* was selected for anti-hypertensive and calcium channel blocking effect. The result of the present study concludes the n-hexane leaves fraction if this plant has potential anti-hypertensive and calcium channel blocking effect. The results were comparable to that of standard drug verapamil. Further studies are needed to explore the exact phytochemicals responsible for antihypertensive effect. Further spectroscopic and chromatographic techniques are needed to determine the exact structure and isolation of these phytochemicals responsible for such activity.

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